Production of Rosemary Oleoresin Using Supercritical Carbon Dioxide

By Fernando Tateo, Maurizio Fellin, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università di Milano, Italia, and Ennio Verderio, Flavourist, Associazione Farmaceutici Industria

An outline has been reported of the experiments helpful to appraise current understanding of extract production with supercritical CO_2 for use in flavourings and fragrances.[1] A paper by the same authors referred to the results of experiments performed on coffee, rosemary, juniper, and celery.[2] Additionally, a recent publication detailed the results of preliminary experiments performed to assess the suitability of employing supercritical CO_2 in the production of oleoresins from *Rosmarinus officinalis* L.[3] From the gas chromatography analysis it follows that:

- The extract obtained with supercritical CO_2 has a nonoxygenated terpenes content which is considerably lower than that for essential oil produced by steam distillation.
- The value of the ratio oxygenated terpenes/nonoxygenated terpenes defined as "D.D." (Deterpenation Degree)[4] is considerably greater for an extract obtained with supercritical CO_2 than that obtained for an essential oil.
- The content of some oxygenated components (1,8-cineole, α-terpineol, d-linalool), taken as indices of comparison among extracts, appears to be decidedly greater in the extract obtained using supercritical CO_2.

The extraction solvents to be used in accordance with good manufacturing practice appear codified in the latest EEC Directive N. 7261/87 of June 22, 1987. This directive concerns the extraction solvents for the treatment of raw materials, foodstuffs or of the components or the ingredients of foodstuffs. The extraction solvents are propane, butane, butyl acetate, ethyl acetate, methanol, propanol-2, CO_2, acetone, and nitrous oxide.

The maximum acceptable levels of some solvents as residues in foodstuffs or the ingredients thereof were stated exactly in the same EEC Directive. The restrictions relate to solvents such as hexane, methyl acetate, methyl ethyl ketone, and methylene chloride (see Table I). The EEC Council has set the maximum limits for the content of residual solvents in foodstuffs, according to the use of the extraction solvents in the preparation of artificial flavourings (see Table II).

In the case of some vegetable products characterized by volatile, degradable fractions, the extraction by solvent leads to final products distinguished by the absence of typical characteristic notes. Sometimes, extraction by organic solvent leads to oleoresins which are not distinguished by the freshness and typical note of the raw materials from which they have been derived.

The advisability of adopting an extraction process apt to lead to a derivative having the aromatic typicality of rosemary leaves is ever present. The various experiments conducted using high pressure extraction (H.P.E.) technology and reported in this paper aim to check the possibility of producing an oleoresin which is rich in typical volatile components and nonvolatile components.

In the case under consideration, the experiments were performed using Tuscan rosemary whereas the experiments discussed in connection with the previous paper[3] were performed using rosemary originating in Lombardy. Table

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Table I. Extraction solvents for which EED Directive N° 7261/87 of 22nd June, 1987 specifies the conditions of use

Name	Conditions of use (short description of use)	Maximum tolerated residue in listed food or in its ingredient
n-Hexane	-Production or fractionation of fat and oils and cocoa butter production	5 mg/Kg in the fat or oil or cocoa butter
	-preparation of proteinaceous products and defatted meals	10 mg/kg in the foods containg the proteinaceus product or the defatted meals
	-preparation of defatted cereals germ	5 mg/Kg in the defatted cereals germ
	defatted soybean products	30 mg/Kg in the soybean products sold to final consumer
Methyl acetate	-Decaffeination or elimination of irritating and bitter substances of coffee and tea	20 mg/Kg in the coffee or tea
	-production of sucrose from treacle	1 mg/Kg in the sucrose
Methyl Ethyl-Ketone	-Fractionation of fats and oils	5 mg/Kg in the fat or oil
	-decaffeination or elimination of irritating and bitter substances of coffee and tea	20 mg/Kg in the coffee or tea
Methylene chloride	-Decaffeination or elimination of irritating and bitter substances of coffee and tea	10 mg/Kg* in the tasted coffee and 5 mg/Kg in the tea

(*) This value will be reduced to 5 mg/Kg three years after the adoption of this Directive.

III details a comparison of data relating to the composition of essential oils derived from the two types of rosemary used in previous experiments and those relating to this paper. Our experiments also showed that the rosemary essential oil's composition varies considerably according to the region of production.

On the other hand, literature available on individual "labiatae" still has to consider "lot to lot" variations because it is very difficult, from a botanical standpoint, to distinguish between the common species. The examination of composition data for rosemary oils reported in a paper by Rhyu[5] clearly shows a substantial difference in the content of α-pinene and 1,8-cineole (eucalyptol) between *R. officinalis* and other labiatae. In essential oils of rosemary, without distinction of origin, the two components cited previously are quantitatively predominant.

The composition data of four samples of rosemary of different origin according to Rhyu are presented in Table IV. Examination of this data also reveals marked variations between products of different origin.

This article intends to appraise the results of adopting different H.P.E. conditions in the extraction of rosemary oleoresins for the purpose of determining the parameters required to obtain the best quality oleoresin.

Apparatus

Gas chromatography

Carlo Erba H.R.G.C. Mega Series
Column: OV 1 (25 M x 0.32 mm I.D.; fused silica, film thick. 1 micron)
Integrator: Chromatopac C-R3A Shimadzu

High pressure extraction—The Nova Swiss—Effretikon "lab" plant consists substantially of a compressor group that pumps the extractant CO_2 at constant temperature from a storage tank, via heat exchanger, to an extraction vessel (capacity 200 ml) equipped with two sintered steel disks at the extremities to allow passage of fluid. Extraction pressure can be varied between 100 and 900 bar. The expansion section where extract recovery takes place is connected through a hand-operated valve.

Essential oil extractor—The model of extractor used is described in the AOAC methods for the separation and the determination of the essen-

Table II. Maximum quantities of residual solvents tolerated in foodstuffs according to EEC Directive N° 7261/87 of 22nd June, 1987

Name	Tolerated residue
Diethyl ether	2 mg/Kg
Isobutane	1 mg/Kg
n-Hexane	1 mg/Kg
Cyclohexane	1 mg/kg
Methyl acetate	1 mg/Kg
1-Butanol	1 mg/Kg
2-Butanol	1 mg/Kg
Ethyl Methyl Ketone	1 mg/Kg
Methylene Chloride	0.1 mg/Kg
1-Methyl-1-Propanol	1 mg/Kg

These residual substances are clearly defined in Part III of the EEC Directive as being derivable from the use of extraction solvents in the production of flavourings made by natural flavours.

Table III. Comparative content of some terpenes in two essential oils of *"Rosmarinus officinalis L."* of different origin

Components	Essential oil	
	A	B
α-pinene	13.5	31.8
camphene	2.6	2.9
sabinene	0.9	0.6
β-pinene	2.1	2.1
myrcene	1.2	1.4
p-cimene	1.1	0.6
1,8-cineole	10.7	18.0
d-limonene	3.9	3.7
linalool	0.9	0.5
α-terpineol	9.3	12.8
Σ	46.2	74.2
Other components	53.8	25.6
D.D. = T.O./T	2.2	1.3

$$\text{D.D. = Deterpenation Degree} \quad \frac{\text{Oxygenated terpenes (T.O.)}}{\text{non-Oxygenated terpenes (T)}}$$

(A) = Autoctonous Lombardy (Lecco district)
(B) = Autoctonous Tuscan (Arezzo district)

Table IV. Terpenes composition of *"Rosmarinus officinalis L."* essential oils from different origin (acc. to H. Y. Rhyu)

	France	Portugal	Spain	Iugoslavia
α-pinene	17.2	6.9	24.5	15.6
camphene	6.8	2.4	9.9	3.2
β-pinene	0.6	1.5	0.7	0.2
sabinene	-	-	-	-
myrcene	2.6	19.1	5.1	1.9
α-phellandrene	0.1	1.8	0.1	0.1
α-terpinene	0.7	0.7	0.5	1.2
d-limonene	3.4	5.4	4.3	3.3
1,8-cineole	20.5	14.7	19.5	18.5
γ-terpinene	1.7	1.8	0.2	0.3
p-cimene	2.7	3.7	2.7	2.0
terpinolene	0.1	0.6	0.1	0.2
thujone	0.3	0.5	0.1	0.5
d-camphor	0.1	0.2	0.1	0.6
copane	0.1	0.3	-	0.3
linalool	17.3	14.4	14.1	14.5
terpinen-4-ol	1.2	1.7	1.7	1.8
β-caryophyllene	1.7	2.9	0.9	2.9
methyl chavicol	0.2	0.3	0.2	0.3
α-terpineol	3.0	3.6	1.8	2.8
thymol	0.7	0.5	-	1.2
carvacrol	0.1	0.5	-	2.0
Σ	81.1	83.6	85.6	73.4
other	28.9	16.4	24.4	26.6
monoterpene	37.7	47.2	45.1	31.2
other components	62.3	52.8	50.9	68.8

Table V. Gas chromatography analysis of *"Rosmarinus officinalis L."* extracts produced with different "P" and "T" parameters (data expressed as rel. percentage)

Extract Components	CO_2 - H.P.E. Extract				
	150 bar/25°C	200 bar/35°C	300 bar/35°C	300 bar/45°C	400 bar/35°C
α-pinene	11.6	19.3	18.1	23.7	18.7
camphene	1.6	2.0	1.8	2.0	1.7
sabinene	0.4	0.3	0.3	0.4	0.4
β-pinene	1.1	1.5	1.6	1.6	1.5
myrcene	0.7	1.1	1.2	1.2	1.1
p-cimene	0.8	0.9	0.8	0.6	0.8
1,8-cineole	17.3	17.5	18.9	17.4	18.5
d-limonene	1.4	2.1	2.0	2.0	1.9
linalool	2.1	1.5	1.5	1.1	1.4
α-terpineol	21.4	19.9	21.6	19.6	20.9
Σ	58.4	66.1	67.8	69.6	66.5
Other components	41.6	33.9	32.2	30.4	33.5
$\frac{\text{1,8-cineole}}{\alpha\text{-pinene}}$	1.49	0.91	1.04	0.73	1.01
$\frac{\text{1,8-cineole}}{\text{d-limonene}}$	12.36	8.33	9.45	8.70	9.63
$\frac{\text{linalool}}{\alpha\text{-pinene}}$	0.18	0.08	0.08	0.05	0.08
$\frac{\text{linalool}}{\text{d-limonene}}$	1.50	0.71	0.75	0.55	0.74
$\frac{\alpha\text{-terpineol}}{\alpha\text{-pinene}}$	1.84	1.03	1.19	0.83	1.14
$\frac{\alpha\text{-terpineol}}{\text{d-limonene}}$	15.26	9.48	10.80	9.60	11.00

tial oils content. The method of steam distillation and subsequent recovery of the oil from the water emulsion by decantation was adopted.

Materials and methods

Gas chromatographic analysis—The gas chromatographic examination of essential oils, the extract with the solvent CH_2C_{12} and the H.P.E. extracts, was executed using the following operating parameters:

Column: OV 1 (25 M x 0.32 mm I.D.; fused silica, film thickness 1 micron)
Oven temp.: isotherm 50°C for 10 minutes, programmed from 50°C to 220°C at 3°C/minutes, isotherm 220°C for 25 minutes
Det. temp. (FID): 280°C
Inj. temp.: 280°C
Carrier: H_2 (2.5 ml/min)
Split ratio: 1/74
Vol. inj.: 0.3 μl

The samples were dissolved in ethyl alcohol 95° at a concentration of 10% (w/v) and filtered by Nucleopore membrane 0.22 micron before the injection.

Table VI. Comparative gaschromatography data between a good sensory quality H.P.E. of *"Rosmarinus officinalis L."* extract and the one obtained with the use of solvent

Components and ratios	Oleoresin (CH_2Cl_2)	H.P.E. Extract (300 bar/45°C for 60 min)
α-pinene	25.5	23.7
camphene	2.4	2.0
sabinene	0.5	0.4
β-pinene	2.0	1.6
myrcene	1.4	1.2
p-cimene	0.6	0.6
1,8-cineole	18.6	17.4
d-limonene	2.5	2.0
linalool	1.0	1.1
α-terpineol	18.7	19.6
Σ	73.2	69.6
Other components	26.8	30.4
$\frac{\text{1,8-cineole}}{\alpha\text{-pinene}}$	0.73	0.73
$\frac{\text{1,8-cineole}}{\text{d-limonene}}$	7.44	8.70
$\frac{\text{linalool}}{\alpha\text{-pinene}}$	0.04	0.05
$\frac{\text{linalool}}{\text{d-limonene}}$	0.40	0.55
$\frac{\alpha\text{-terpineol}}{\alpha\text{-pinene}}$	0.73	0.83
$\frac{\alpha\text{-terpineol}}{\text{d-limonene}}$	7.48	9.60

High pressure extraction—In the experiments executed using the "lab" plant, 85 g of *R. officinalis* leaves were subjected to extraction each time (1 h batches). The extraction material was not ground beforehand; thus the volatilization of several aromatic principles was prevented. The discharge stage was set at 20 minutes, at the same extraction pressure and at a flow of 2 g CO_2/h. Five extraction operations were executed under different conditions of temperature, "T" and pressure, "P" using the "lab" plant as follows:

T (°C)	P (bar)
25	150
35	200
35	300
45	300
35	400

It should be remembered that CO_2 is in a supercritical state at temperatures and pressures exceeding 31.2°C and 73.9 bar. It follows that the parameters 25°C and 150 bar correspond to CO_2 in the liquid state.

Table VII. Extractions yield under different operating conditions

H.P.E. parameters	Extract yields (%)
150 bar/25°C for 60 min	1.5
200 bar/35°C for 60 min	2.1
300 bar/35°C for 60 min	6.1
300 bar/45°C for 60 min	5.3
400 bar/35°C for 60 min	4.3

Table VIII. Deterpenation degree for different CO_2-H.P.E. extract of "*Rosmarinus officinalis L.*" The data are compared with those corresponding to the essential oils prepared from fresh material and with the oleoresin produced by solvent (CH_2Cl_2)

Extract components	CO_2 - H.P.E. Extract					Oleoresin (CH_2Cl_2)	Essential oil
	150 bar/25°C	200 bar/35°C	300 bar/35°C	300 bar/45°C	400 bar/35°C		
Terpenes (T)	18.6	29.0	26.1	32.5	26.3	35.6	44.3
Terp. oxygenated and others (T.O.)	81.4	71.0	73.9	67.5	73.7	64.4	55.7
Σ	100.0	100.0	100.0	100.0	100.0	100.0	100.0
D.D. = T.O./T	4.4	2.5	2.8	2.1	2.8	1.8	1.3

Extraction of oleoresins by solvent—The leaves of *R. officinalis* were extracted by solvent (CH_2C_{12}) by stirring in a closed flask at 25°C for 1 h with a "solids/liquid" ratio 1:5. After filtration, the solvent was eliminated by evaporation in vacuo at 30°C.

Test Results

The gas chromatography data relating to some of the terpenes imparting distinctive properties to H.P.E. extracts produced in the conditions reported in "materials and methods" and the values of some ratios considered useful for comparison are reported in Table V. The composition of the H.P.E. extract with the best sensory quality compared with that of the oleoresin produced using solvent (CH_2C_{12}) is reported in Table VII. In reality these two derivatives are very similar from the sensory quality viewpoint. The HRGC chromatogram of the extract produced at values of P = 300 bar and T = 35°C appears in figure 1.

For the identification of the optimum operating conditions for H.P.E. extraction, it is also important to report the indicative data for percentage extract (oleoresin) yield. Table VI contains the percentage yield data, the values of which clearly relate to fresh vegetable materials. The extraction yield reaches the maximum value of approximately 6% under conditions of pressure and temperature of 300 bar at 35°C for 60 minutes which were also adopted in earlier experiments.[3] The extract yield was a minimum at 150 bar at 25°C for 60 minutes.

From the gas chromatographic findings shown in Tables III, V and VI, we may deduce what is set forth in Table VIII concerning the "Deterpenation Degree" (D.D. = oxygenated terpenes/nonoxygenated terpenes and others). A D.D. value exceeding that of the oleoresin produced using solvent and to that of the corresponding essential oil is found with all H.P.E. extracts. The most deterpenated product is that

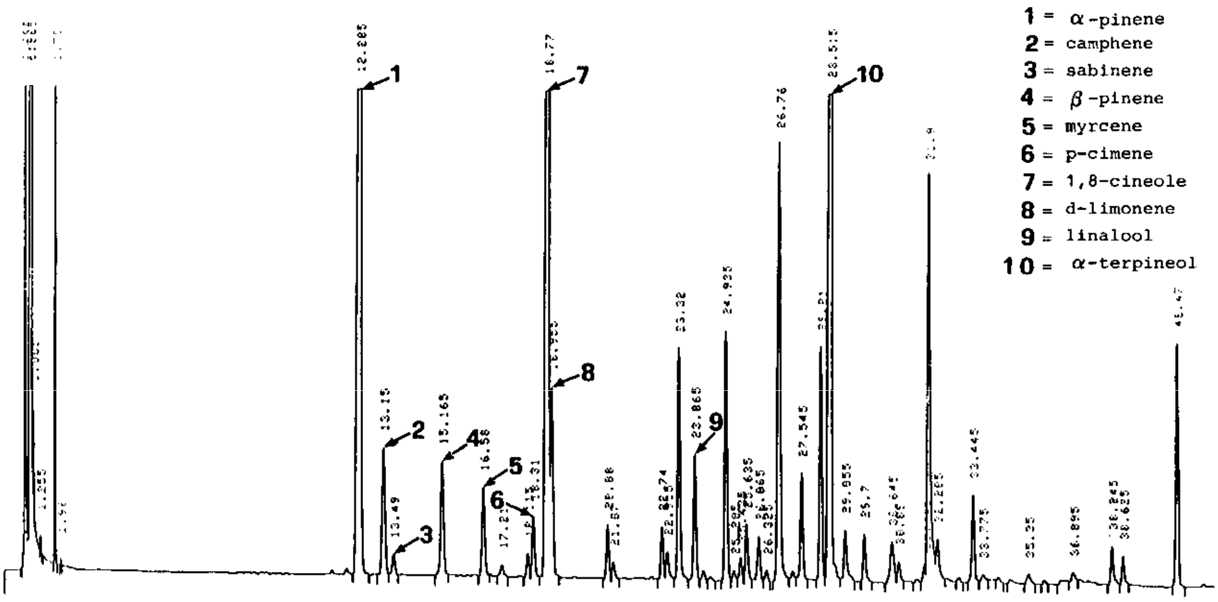

Figure 1. HRGC chromatogram of supercritical HPE-CO_2 extract (300 bar/35° C/60 min) from *"Rosmarinus officinalis L."*

produced with liquid CO_2 (150 bar at 25°C for 60 minutes).

Conclusions

H.P.E. CO_2 technology permits preferential extraction of α-pinene in supercritical fluid conditions more than in liquid fluid conditions. This is deduced from the values of oleoresin composition shown in Table V. The oleoresin produced at 150 bar at 25°C for 60 minutes results in a lower α-pinene content, and this causes the appearance of a fatty-like note. The extract possessed the lowest α-pinene content of any of the extracts produced.

The optimum H.P.E. conditions are those corresponding to the parameters 300 bar at 35°C for 60 minutes and 300 bar at 45°C for 60 minutes at which greater extraction yield also occurs.

Concerning the sensory quality properties, the oleoresins obtained with H.P.E. parameters—300 bar at 35°C for 60 minutes and 300 bar at 45°C for 60 minutes—proved to be particularly true to the aroma typical of the fresh leaf fragrance of rosemary. The quality of the extract obtained with parameters 400 bar at 35°C for 60 minutes as a whole was slightly lower than that for extracts obtained at 300 bar even though this also has a good note reconducible to the aroma of fresh rosemary leaves.

Finally, the oleoresin obtained with the parameters 150 bar at 25°C for 60 minutes is distinguished by a "fatty" note which makes that derivative organoleptically poorer than the others. However, the basic aromatic typical of rosemary were present in this extract also.

Concerning any economical factors of the H.P.E. process, it is fundamental to state that the deterpenation process is a consequence of the extraction process, without additional costs. The H.P.E. process also constitutes, in the case of *R. officinalis*, a useful technology for the preventive treatment of vegetable material.[6]

References

Address correspondence to Prof. Fernando Tateo, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche—Cattedra di Aromatizzazione dei Prodotti Alimentari, Università di Milano, Via Celoria, 2, 20133 Milano, Italia.

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